DEVELOPMENT OF A HIGH DENSITY PERCUTANEOUS CONNECTOR SYSTEM

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Abstract

This report summarizes activity over the period from April 15, 1999 through July 15, 1999 on NIH Contract N01-DC-7-2103, "Development of a High Density Percutaneous Connector System". During this quarter the final deliverables were determined and implants initiated and leakage problems were investigated. A quick disconnect (QD) design has been developed.

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I. Background and Review of Contract Requirements

This report summarizes activity during the specified quarter, on NIH Contract N01-DC--2103, "Development of a High Density Percutaneous Connector System". Over the course of this contract, a high density, planar, low profile connector system is being developed that incorporates pad grid array technology. This technology has unique advantages as applied to a percutaneous interconnect system. In particular the connector system will be low in profile, easy to clean, sealed against ingress of contaminants, offer low mechanical resistance to mating and demating and provide a very high number of contacts in a small diameter. The connector system will be implanted in a suitable animal model and the appropriate electrical, mechanical, and biocompatible properties of the system will be assessed. The specific technical requirements of this connector system as detailed in the contract are explained below:

- The connector will incorporate a pedestal that can be attached to the skull in a mechanically stable manner. The pedestal will be designed to accept a replaceable connector assembly. All materials of the pedestal in contact with tissue will be biocompatible and the profile of the pedestal will be low enough to minimize any physical trauma during mating and demating of the connector or due to normal physical activities.

- The connector assembly will be high-density with at least 70 contacts. The electrical isolation between the contacts or between the contacts and the body should withstand at least 18 volts without breakdown. The connector contacts when mated should be capable of passing up to 20 mA of current with less than a 1.0 volt drop across the connection. A simple method of mating and demating the upper and lower surfaces of the connector should be provided. In addition, a convenient means to attach electrical leads to the connector is needed.

- The connector will be designed from materials that are durable and can withstand the physical abuse from normal activities of daily living. The interface between the connector and the skin must be such that the passage of microorganisms into the body and fluid drainage out of the body is prevented.

- In earlier studies connectors had five separate loops of insulated wire, each 2 inches long. Because of wire breakage observed during these studies, it is necessary to make a more durable and a more realistic part. The present cable is a ribbon one-inch long with five 2-mil Pt Ir wires, coated with Parylene and Silicone. The wires are looped so there are five loops for testing. The 2 mil wires are more rugged and easier to work with for initial tests, but 1 mil wires will be used after the ribbon cable concept is developed. An 18-Volt bias will be maintained on the wires relative to an implanted platinum wire connected to one of the unused contacts or the Ti connector body. The leakage current of the cable wires will be monitored with a maximum acceptable value of 10 nanoamperes.

- Performance of the connector system will be tested in a suitable animal model. After three to six months of implantation, the connector assembly will be explanted and gross and microscopic examinations will be performed to study the attachment of the pedestal to the skull, the attachment of the skin and soft tissue surrounding the pedestal to the pedestal wall and the reaction of adjacent tissue to the implanted device.

- Finally, design changes and improvements, if needed, will be recommended. A set of connectors will be fabricated and sent to the NIH for implantation. Initial testing will be in cats with final tests conducted in non-human primates.

II. HMRI Work

For six animals (TC36 to 41) HMRI reported that infection was "considerably reduced compared to what we reported in previous QPRs". Tc36 is a grooved Titanium surface with Laminin-5 added (at the University of Washington Engineered Biomaterials - funded by the National Science Foundation). Tc37 - 41 are Titanium beaded surfaces and were electrostimulated a 1 mA bipolar for three hours per day. The beaded surface may have contributed to the reduced infection level although skin attachment was poorer than expected, perhaps because the sintering of the beads caused features that were more "lump-like" than bead-like. (The vendor "lost" the process for small parts such as these. Things have improved in recent months, but are not yet back to the 1998 quality.) The grooved Titanium surface with Laminin-5 had good skin attachment and reduced epithelial downgrowth. This is a marked improvement over the first Laminin-5 implant in which the skin was healthy but apparently unattached on gross examination (histology showed attachment).

Osseointegration was reported to range from 23% to 100% with an average in three of the cats of 83.5%. Electrostimulation was found to improve osseointegration and to align fibroblasts at the skin attachment surface in two of three animals stimulated. HMRI reports that, "Although encouraging, ...the data suggest that it may be premature to make a definitive correlation between electrical stimulation and skin attachment, osseointegration or both". Details are in the attached HMRI QPR (Appendix 1).

The HMRI QPR makes repeated references to needing "...good coverage of the undersurface of the BP (base pedestal) with Titanium beads". Each pedestal was completely covered with a bilayer or more of Titanium beads. However, close examination of the surface shows spots where a few beads are missing forming pockets. The presence of these pockets is being minimized, but will probably not reach zero.

A more serious problem with osseointegration was listed at the top of HMRI page 3: "...variability in the convexity of the sculpted surface of the skulls...." The tool used to shape the skull sits at three points on the skull and is consistent to the degree to which this position can be held for five or ten seconds. The mating surface, the bottom of the base pedestal, is checked with a gauge before shipment to HMRI. The skull to Titanium bead surface gap must

be small enough that the bone can "sense and jump to" the Titanium. Earlier pedestals had gaps of up to 0.02 inches which is much too large. The present parts are held to about one-half the bead diameter or 0.003 inches.

III. Leakage Problems

In the last QPR it was reported that.

- The leakage is in the lower connector section (LCS), not the cable
- The long term leakage is less than 50 nA if proper cleaning and passivation of the Titanium are used
- The long-term leakage is not observed in implants because of the relatively short times of implant
- The possible physical locations of electrical leakage were discussed as well as the possible sources of ions to provide the conduction mechanism(s)
  - Diffusion of ions through the epoxy pin matrix
  - Diffusion of ions along the gap at each pin
  - The Alumina used to align pins

Five Figures are present below showing the results of recent leakage tests. These are selected from a larger data set to illustrate the findings. Figure 1 shows the configuration using Platinum pins, the less pure Alumina and the Alumina is not leached. This is the control which is similar to the connector design used for several years. Note that the current remains below the 10 nA level. Figures two and three are for Platinum pins with a more pure Alumina (to reduce ions from this source) and with the Alumina leached by boiling in DI water for one hour to remove ions. In both cases the current is greater than that shown in Figure 1, the control. The reason for the increase is not clear, but the Alumina is not the source of ions.

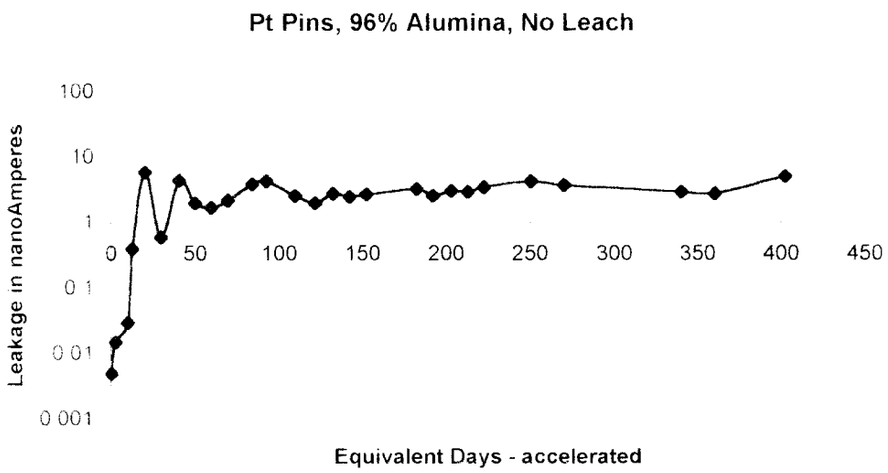


Figure 1. Electrical leakage in the Control.

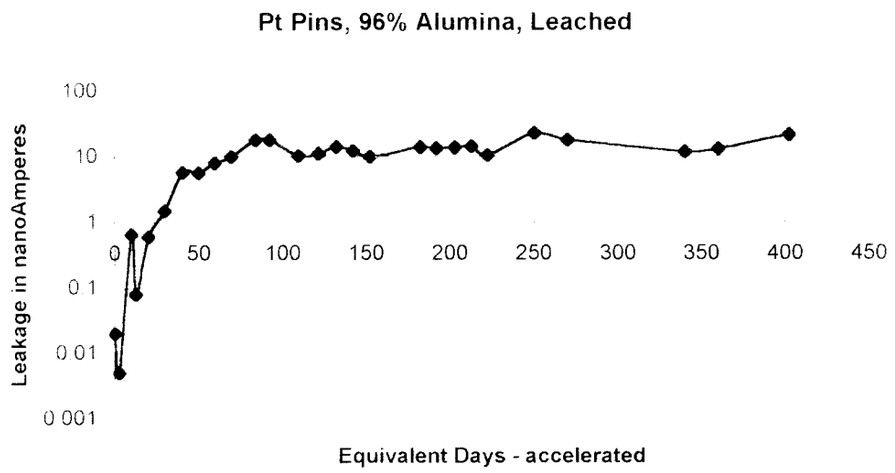


Figure 2. Electrical leakage when the less pure Alumina is leached by boiling.

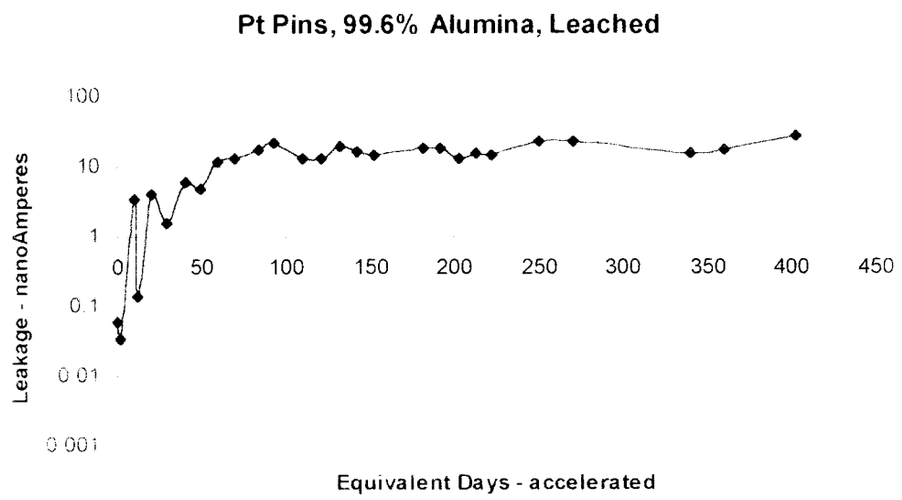


Figure 3. Electrical leakage on a more pure Alumina with leaching.

From prior work on explanted connectors it is well established that the gap between the Alumina pieces is a significant source of leakage if not the only one, because it is almost impossible to fill completely with epoxy. This leaves air gaps where moisture and ions can collect causing leakage. Past experience has shown the pin matrix epoxy is inherently capable

of much lower leakage levels and is not the ion source. Therefore ions that cause the leakage are most likely from the Ringers solution (or body fluids) diffused through the epoxy or along microscopic gaps adjacent to the pins into the gap between the Alumina pieces. The cure for this problem is to find a way to fill or remove the gap.

Another more hydrophobic material, Ciba's Arocy XU 366, was used in a similar set of measurements. In all cases it showed orders of magnitude higher leakage. A small amount of solvent was used to reduce the viscosity during fabrication. This would cause shrinkage as the solvent evaporates leaving gaps along the pins that would provide a path for ion movement into the connector. At this time, no conclusions can be drawn with regard to the material itself.

Figures 4 and 5 show leakage for similar devices using Gold pins instead of Platinum. Leakage is much higher. A possible reason is that the epoxy pin matrix will form a bond with an oxidized surface, but not with a clean metal surface. With clean metal leakage control depends on a tight mechanical fit. While Platinum is a relatively stable metal, it can form an oxide. Gold does not. No effort was made to oxidize the Platinum, but it is likely that some oxide was formed and may have improved the epoxy-to-pin seal. In any event, the Gold is a less desirable pin material with respect to electrical leakage in these experiments.

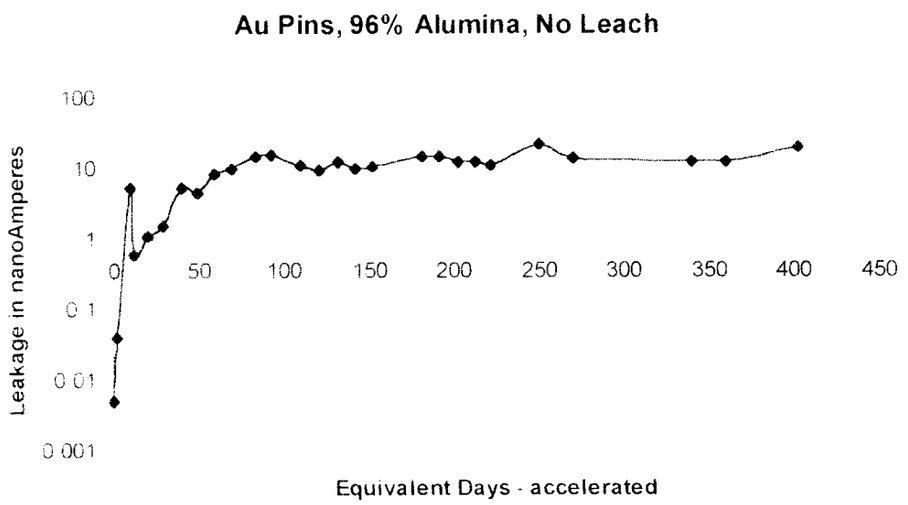


Figure 4. Electrical leakage for a control device using Gold pins.

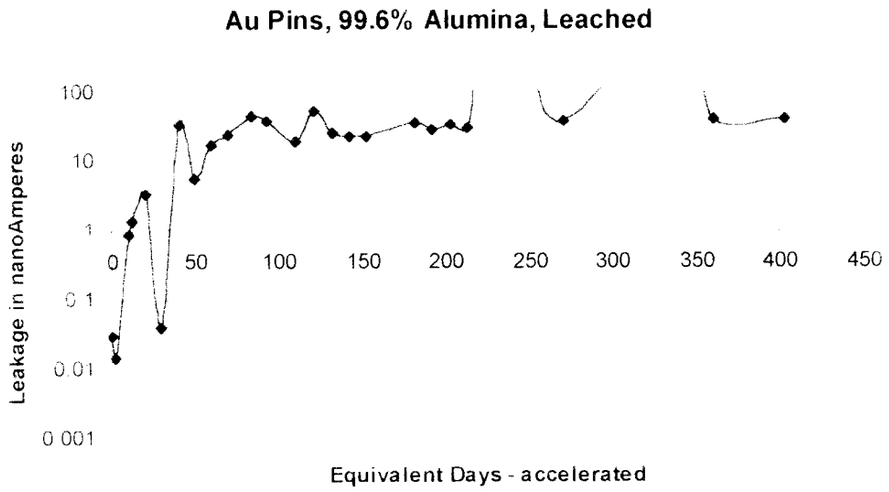


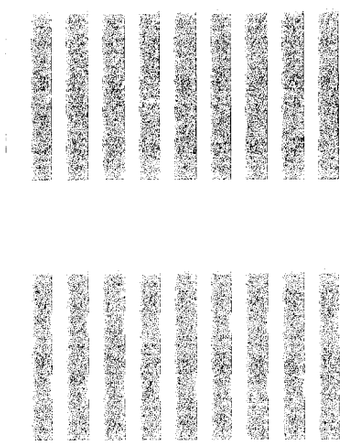
Figure 5 Electrical leakage for a device with Gold pins and leached, more pure Alumina.

Figures 1, 3 and 5 show an interesting phenomena that may indicate the leakage mechanism. All three show a transient oscillatory characteristic in the first 50 equivalent days. Such a characteristic was reported by Torsten Teorell during the 1950s [1-5]. It requires a porous membrane with fixed charges that separates the two solutions of different ions in water and an electric potential. The mechanism involves diffusion, an electric potential and hydrostatic pressure as fluid levels vary on each side of the membrane. The device is referred to as a membrane oscillator and can show transient or steady-state oscillations. The literature shows oscillation periods of minutes to hours. Teorell suggested a connection with action potentials in the nervous system and similar phenomena that are on the order of milliseconds in the body. In our case the periods are days or weeks indicating some combination of lower diffusion rates, lower electric potentials and lower hydrostatic pressure; all are probable. Although this mechanism is conjecture for the percutaneous leakage, the data strongly suggests this or a similar mechanism since diffusion alone does not oscillate.

Elimination or filling of the gap between the Alumina pieces was given above as a method to reduce leakage significantly. Figures 6 show cross sections through the centers of the old and a possible new percutaneous connector. Measurements will be made on the new connector during the next quarter.

In Figure 6a the Titanium body is shown on the left and right with a small ledge on which two pieces of Alumina rest. Nine of eighty-one pins are shown vertically, positioned by holes in the horizontal Alumina. The space above and below the Alumina is filled with modified EpoTek 301 (M301). The gap between the Alumina pieces should also be filled with

M301, but this is almost always less than perfect leaving air gaps in which electrical leakage can occur.



(a) Old Construction showing Alumina and gap between pieces of Alumina.

(b) New construction without Alumina or gap.

Figure 6. A comparison of the old and potentially new percutaneous construction.

Figure 6b shows the newer construction in which a piece of M301 or other polymer has holes drilled for pins. This piece is then inserted into the Titanium, pins are inserted and M301 is used to fill gaps and secure the polymer block to the Titanium. The gap is eliminated. This is relatively easy to build for a laboratory experiment, but if it is successful practical manufacturing methods will have to be developed.

V. Investigation into Other Pin Matrix Materials

The tests and results reported in the last three QPRs continue without significant change. No additional tests are expected to start until current work on the leakage problem is completed.

VI. Skin Growth and Attachment

As reported in the last QPR, Dr. Dave Edell has pointed out that skin attachments have a history of failure at three to four months. Long-term implants (4 to 6 months) have been

started at HMRI. These implants use the beaded skin attachment surface that has proved successful during the last year.

The graded interface described in the last QPR was not implanted because we have not fabricated the final implant devices. These wait on the results of the leakage tests are expected to be done this fall.

VII. Quick Disconnect

The Quick Disconnect (QD) design has been completed and fabrication is expected to start in the next quarter. It is not expected that the QD connector will be implanted during this contract.

VIII. Primate Studies

There has been no change in the intention to complete three primate implants. We are waiting on our sister project to complete work necessary for the implants to progress.

IX. Activities for the Tenth Quarter

During the next quarter:

- Continue to reduce leakage
- Continue long-term skin attachment implants (4 to 6 months)
- Develop the graded interface design and manufacturing methods for the interface
- Implant at least one graded interface with a beaded surface
- Continue the accelerated life testing of polymers for use as pin matrix materials
- Fabricate the Quick Disconnect design
- Continue to assist with non-human primate work

References:

1. Teorell, T., *Exp. Cell Research*, 1955, suppl. 3, 339.
2. Teorell, T., *Z. Physikal. Chemie.*, 1958, 15, 25.
3. Teorell, T., *Exp. Cell Research*, 1958, suppl. 5, 83.
4. Teorell, T., *Gen. Physiol.*, 1959, 42, 4, 831.
5. Teorell, T., *Gen. Physiol.*, 1959, 42, 4, 847.

Appendix I

HMRI Report

Development of a Percutaneous Connector System

Quarterly Progress Report Number 9

August 1, 1999

Subcontractor

Huntington Medical Research Institutes

Neurological Research Laboratory

Pasadena, California

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Summary

In this quarter, we present the results of six animals (TC-36-TC-41). In this series of experiments, infections along the sides of the first connector stage (FCS) and base pedestal (BP) portions of the connectors was considerably reduced compared to what we reported in previous QPRs. Skin attachment to the FCS was good in 1 of 5 cats and attachment to the BP was good in 5 of 5 cats. In TC-38, the sixth cat, the FCS was removed several weeks after implanting due to excessive tissue injury. In this animal, skin attachment was poor at the BP. Mixed leukocytes (mononuclear cells, macrophages, and a few lymphocytes and neutrophils) were observed at the sides of the BP in TC-38 and within the interface between the BP and skull. Several small foci of mixed leukocytes were observed distant from the connector in TC-39. Although there was reduced infection of the soft tissues adjacent to the connectors, soft tissue was not attached to the FCS despite the fact that connectors implanted in 4 of 6 animals had been sinterred on the sides. This was probably related to marsupialization of the skin, since there was good attachment to the ground sides of the BPs, in 5 of 6 cats. Skin marsupialization was present in 4 of 6 animals. Addition of Laminin-5 in one animal (TC-36) appeared to reduce marsupialization and improve soft tissue attachment to the FCS.

In these six animals we noticed variability in the amount of osseointegration ranging from 23% to 100%. In 3 of 6 cats, average osseointegration was 83.5% while in the remaining 3 cats, osseointegration averaged 30%. Improved osseointegration was observed in 2 of 3 animals in which 1 mA of A. C. current (50 Hz) was chronically passed between the connector and a loop of platinum wire in the soft tissues. The stimulation was administered for 3 hrs/day. In TC-40 and TC-41, fibroblasts oriented in a radial fashion with respect to the metal connector were observed along the sides and also under the BP. Osseointegration was excellent in TC-36, TC-37, two cats in which electrostimulation was not performed. Although encouraging, based on six cats in this series, the data suggest that it may be premature to make a definitive correlation between electrical stimulation and skin attachment, osseointegration or both. The

differences in the amount of osseointegration may also be a reflection of: 1) variability in the convexity of the sculpted surface of the skulls, and 2) variability in sinterring of the titanium beads located on the undersurface of the BPs. The best BP-to-skull attachments occurred in those BPs in which the underside of the BP was completely covered with beads. Conversely, a poor match between the skull and the BP surfaces resulted in the least osseointegration (TC-38). Moreover, in TC-41, the undersurface of the BP was not well covered with beads and, despite a good fit between the concave (metal undersurface) and convex (skull) surfaces, osseointegration was only 28%.

Based on the observations presented here, several factors appear to improve osseointegration of the BP: 1) proper sculpting of the skull with the custom drill bit, so as to produce an intimate contact between the undersurface of the BP and skull, 2) good coverage of the undersurface of the BP with titanium beads, 3) reducing soft tissue infections around the sides of the percutaneous connectors, 4) addition of Laminin-5 to the sides of the FCSs, and 5) application of low level electrostimulation to attract fibroblasts to the sides of the FCS and BP to improve soft tissue attachment to the sides of the connector.

Introduction

The objective of this project is to develop a percutaneous connector with a high contact density. Devices are currently under development that are expected to exhibit good biocompatibility and optimal BP osseointegration, and with adequate soft tissue attachment to the FCS and BP. Some of the approaches that we will examine in order to reduce skin marsupialization and soft tissue infection and encourage better skin attachment to the connectors and improve osseointegration, include the addition of Laminin-5, homologous immunoglobulins to retard soft tissue infection, the addition of other related growth factors, the application of sintered titanium beads to the sides of the FCS and BP and the use of low level AC electrical stimulation for 3 hrs/day. We implanted one Laminin-5-coated connector (TC-36), prepared by Bioelectric Corp., Portland, OR. Laminin-5 was added to the grooved titanium sides of the FCS. One of the main objectives of these studies was to further evaluate the effect of adding

sintered titanium beads to the sides of the FCSs and the effect of low level electrical stimulation on soft tissue attachment.

Percutaneous connectors from two monkey experiments conducted at NIH were evaluated histologically in our laboratory. Findings suggested that if the undersurfaces of the BPs are flat with limited beaded surfaces, osseointegration was poor. We have made considerable improvement in osseointegration with the addition of sinterred titanium beads to the undersurfaces of the BP. Based on recent experiments, the addition of titanium beads to the surfaces of FCS and BP stages appears to improve attachment of soft tissue to the sides of the FCS and BP. These data, and the results of the experiments described in this report demonstrate methodology for improving osseointegration in the feline percutaneous model.

Methods

Percutaneous Connector Design. The percutaneous connector has been described in detail in previous QPR's. The percutaneous connector consists of a BP composed of titanium metal. The underside of the pedestal is coated with sintered titanium beads, and the sides have circumferentially milled grooves, ca. 100 µm deep and 125 µm wide. The beads and grooves have been designed to facilitate the attachment of bone, skin and muscle tissues to the connector. The lower (percutaneous) stage contains the pin grid array, pin-wire bonds, silicone and epoxy potting surrounded by a titanium ring with circumferential grooves approximately 20 µm deep and 40 µm wide. The FCS, i.e., the mating system, traverses the skin line. The configuration of the FCS was slightly different in each of the five cats. In TC-36, Laminin-5 was added to the sides of the FCS prior to its arrival at HMRI in an attempt to encourage better soft tissue attachment to the grooves of the connector, and thus, prevent infection. The sides of the FCSs in TC-37 - TC-39, TC-40 and TC-41 were coated with sintered titanium beads.

Surgical Procedure. After dislodgement of two pedestals implanted in a one stage procedure (included TC-36), we are now using a 2-stage surgery separated by 60 days to increase osseointegration.

First Stage Surgery. Under general anesthesia, a rainbow-shaped skin incision measuring approximately 4 cm was made over the right parietal cranium. The skin and muscles were retracted. A mark was made 16 mm distal to the sagittal fissure and 9 mm from the midline at which point a starter hole was drilled to accept the tip of the major drill, which was used to shape the skull to conform to the bottom of the pedestal. The drill bit worked very well, and, in most cases the pedestals fit the convexity satisfactorily. The retaining screws were inserted into the skull using a torque wrench. The pedestals appeared to be firmly attached to the skull. The muscles were sutured over the pedestal, and to the medial surface of the intact muscles on the right side. The galea was closed as the second layer, bacitracin was added, and the skin wound was closed.

Second Stage Surgery. Under general anesthesia, a rainbow-shaped incision was made approximately 6 mm from the base pedestal on the right hemisphere, taking care to avoid the first stage incision. A hole was cut through the dermis on top of the pedestal and a circular subcutaneous pocket was fashioned to house the ground wire for the electrostimulation experiments. The connector was bolted to the base pedestal and then fitted through the hole in the skin. The ground wire was inserted and secured on top of a muscle layer and not to the bone. The wounds were then flushed with Bacitracin (antibiotic).

Leak Testing/Electrostimulation Series. Bias leak tests were not performed on any of the animals in this group of experiments. Electrostimulation was performed in some animals to test the effect on skin healing of low level (~1 mA), 50 Hz AC current for 3 hrs/day. TC-36 - no stimulation, TC-37 - no stimulation (control, sacrificed after 30 days), TC-38 - no stimulation and the FCS was removed because the cables protruded through the skin, TC-39 - stimulated for ca. 30 days and sacrificed, TC-40 - stimulated for 90 days, TC-41 - stimulated for ca. 30 days and sacrificed.

Vascular Perfusion, Embedding and Sectioning.

Four to 20 weeks after surgical placement of the connectors, anesthetized animals were transaortically perfused with PBS for blood removal followed by ½ Karnovsky's Fixative in 0.1 M Schultz's Phosphate Buffer at pH 7.3. After perfusions,

the animals were decapitated and the heads were placed in the above-mentioned fixative in a cold room or refrigerator for several days at ca. 6-8 °C prior to removal of the connectors. Each connector and associated skin, muscle and bone was removed as a single tissue block using a scalpel and a Lipshaw Bone Saw. These blocks were immersed into fresh fixative for several additional days. All blocks were subsequently embedded in glycol methacrylate and 20-40 µm sections were cut with a Beuhler Saw. Unpolished sections were mounted on glass slides with a rapid bonding adhesive, stained with toluidine blue, cover slipped and examined by light microscopy and photographed.

Results

Autopsy. Gross, necroscopic evaluation of TC-36 revealed that most of the skin and associated soft tissues was attached to the sides of FCS. In TC-37 to TC-41, little soft tissue was attached to the sides of the FCS. Sinterred beads were not added to the FCS in TC-36, but Laminin-5 was added to the FCS. In one small area on the caudal FCS, however, soft tissue was not well attached. In TC-37, TC-39, TC-40 and in TC-41, sinterred beads were added to the sides of the FCSs. In TC-38, the FCS was removed shortly after being implanted because the ground wires protruded through the skin and produced a skin lesion.

Histology.

a. Inflammation of soft tissues and bone. In TC-36, TC-37, TC-39, TC-40 and TC-41, little or no leukocytic infiltrates (mononuclear cells and macrophages) were observed within the subcutaneous tissue, dermis and muscular layer immediately adjacent to the metal connector, or at some distance from the connector. In 5 of 6 animals, leukocytes were not observed within the interspace between the skull and the undersurface of the BP. In TC-38, however, there was soft tissue extending to the metal-bone interspace. These cells consisted of a mixture of stellate fibroblasts and fibrocytes and mixed leukocytes including mononuclear cells, macrophages, as well as small, round lymphocytes and neutrophils (Figures 1, 2). **TABLE I** presents a summary of these findings.

b. Attachment of soft tissues to the sides of the connector. The best examples of good tissue attachment to the FCS was observed in TC-36 (Figure 3), and TC-40 (Figures 12, 13). Otherwise, a common feature among the other animals with FCSs (TC-37, TC-39, TC-40 and TC-41) was variability in the amount of marsupialization (Figures 4, 5, 7). This observation was made in all animals except TC-38, which did not contain a percutaneous connector component. In all animals, the skin was attached to the sides of the BP (Figure 12). Radial streaming of fibroblasts and fibrocytes in the direction of the BP was seen in 2 of 3 cats in which electrostimulation was applied. This phenomenon was also observed in an unstimulated cat (TC-37, Figure 10). Connective tissue was also present within the interface between the BP and surface of the skull in some cases, especially in TC-37 (Figure 6), TC-39 and TC-41.

c. Osseointegration. Similar to other animals which we have reported in previous QPRs, variability in the amount of osseointegration was observed. However, in this series of animals, there appeared to be an improvement in the amount of osseointegration, compared to previous animals. In all animals, the width of the pedestal base-bone interfaces were measured in the histologic sections using an ocular lens scale on an Olympus Light Microscope. Quantitation of osseointegration was based on evaluations of 3-6 histologic sections/animal (cord sections across the connectors). By light microscopy, osseointegration was identified as yellowish appearing osteoid tissue directly attached to the opaque titanium metal. Any evidence of blue stained, fibrotic and/or connective tissue within the bone-metal interface was interpreted as an area where osseointegration did not occur. In TC-36, TC-37 and TC-40, osseointegration was exceptional, averaging 83.5% (Figures 3, 8-15), while in TC-38, TC-39 and TC-41, osseointegration averaged 30%. Average osseointegration for all six animals was 57%. As we have reported previously, in all six cats, the best osseointegration occurred within the recesses between the titanium beads associated with the undersurfaces of the BPs (Figures 9, 12, 16), and also within the threads of the titanium screws used to secure the BP to the skull (Figures 13, 14). The results are presented in **TABLE I**.

TABLE I

Cat No.	ELECTRO-STIMULATION YES/NO	IMPLANT TIME FCS/BP (IN DAYS)	FIBROBLASTS IN A RADIAL PATTERN TOWARDS CONNECTOR	LOCATION OF INFLAMMATION	ATTACHMENT OF SOFT TISSUES TO FCS AND/OR BP	AVERAGE OSSEO-INTEGRATION
TC-36	No	96/96	No	Sides of FCS and adjacent to BP	Good at FCS and at BP Some Marsupialization at FCS.	83%
TC-37	No	44/112	Yes, at BP	Limited at sides of FCS. Good at BP	Attachment only to base pedestal	67.5%
TC-38	No	27/113	No	Limited at sides of FCS	Limited at BP	23%
TC-39	Yes, 30 days	42/105	Minimal at BP	Leukocytes distant from connector	Marsupialization at FCS Good at BP	39%
TC-40	Yes, 90 days	91/143	Yes, at BP	Leukocytes distant from connector	Marsupialization at FCS Good at BP	100%
TC-41	Yes, 30 days	44/96	Yes, at BP and extending to interface between BP and skull.	Minimal leukocytes at sides of FCS	Marsupialization, poor at sides of FCS. Large empty space at one side of FCS. Most of BP good.	28%

Discussion and Future Studies

The most important findings from the six animals include: 1) skin marsupialization and poor soft tissue attachment to the FCS remains a problem in 4 of 6 animals, 2) various degrees of soft tissue infection was observed along the sides of the FCS and the BP, 3) variability in osseointegration was noticed among the six animals evaluated, but in 3 of 6 cats, osseointegration was excellent; in the other three cats, osseointegration was fair. Good osseointegration (defined as greater than 50% bone tissue connected to the undersurface of the BP) was observed in 3 of 6 cats. In three cats, osseointegration averaged 83.5.5% and 30% in the other three animals. The overall average osseointegration for this series of cats was 57%, 4) Laminin-5 appeared to encourage attachment of the soft tissue to the FCS in one animal and 5) electrostimulation appeared to stimulate fibroblasts at the sides of the BP in 2 of 3 cats stimulated, and also appeared to influence osseointegration in 2 of 3 cats.

Skin attachment to the metal connector. Marsupialization appears to be a consistent problem in the feline model. This is a difficult problem since the growing epidermis will tend to wall off a percutaneous structures. Consequently, skin attachment at the epidermis may not occur. Skin movement may also prevent skin attachment while the deeper dermis and subcutaneous tissue may be more stable and, thus, may be a more favorable environment for attachment. This may explain why most of the skin attachment in 4 of 6 animals occurred at the deepest regions of the subcutaneous tissue, ie, at the sides of the BP. In TC-36, the addition of Laminin-5 appeared to improve tissue attachment to the sides of the FCS, whereas, in our previous QPR (TC-32), we reported that this treatment failed.

Soft tissue infection and osseointegration. Another problem associated with percutaneous connectors in the cat, and often associated with marsupialization, is skin and dermal infections. Previous attempts to prevent skin infections have included local application of hypertonic sugar solutions, honey, antibiotics, or a combination of both topically added to the skin surrounding the protruding percutaneous FCS. Unfortunately, these techniques have resulted in little success in the prevention of skin infection. In previous animals, severe infections observed around the sides of the FCS

and BP seem to have inhibited the adhesion of the dermis and subcutaneous tissue to the sides of the metal connector, despite the addition of either grooves or beads to the sides of the FCSs. In previous animal experiments, our results suggested that the dermis and subcutaneous tissues appeared to attach better to the deeply grooved titanium surfaces when infection was not widespread. Thus, it is reasonable to speculate that if widespread infection is present, soft tissue attachment may be prevented, even if deep groves or sintered beads are added to the sides of the FCSs. We reiterate that chronic skin infections may prevent adequate osseointegration due to the local release of proinflammatory cytokines and other proteins by leukocytes. In one previous QPR (TC-32 and TC-34), we reported that in animals in which the skin infection was reduced or limited to the sides of the FCS (TC-36, TC-37, TC-40), osseointegration was considerably improved. The observation presented here confirms that osseointegration occurs optimally within the deep recesses of the sinterred titanium beads to the undersurface of the BP (Figures 8, 11, 15). Numerous animal experiments have demonstrated that:1) excellent ingrowth of osteoblasts occurs within the deep grooves of the anchoring screws, and 2) BPs with flat undersurfaces do not appear to support osseointegration. Adding the sinterred beads with good coverage of the undersurface of the BP is important to insure good osseointegration. This was especially true for TC-40.

Another issue is the variability in the contact between the undersurface of the BP and the concavity made in the skull surface by the shaping drill bit. If a good fit is not established, as in TC-38 (Figure 1), adequate osseointegration may not occur. Conversely, if the abutment between the two opposing surfaces are well matched as in TC-40, excellent osseointegration will occur. Thus, it is likely that careful fitting of these two surfaces is critical for adequate osseointegration.

Inflammatory cells and/or microbial pathogens may release toxic substances that iinhibit the growth of osteoblasts and connective tissue. Further studies will be required for elucidation of this phenomenon. We wish to establish precisely how much osseointegration will be adequate for human application. For our cat experiments, a key question will be to determine how to reduce or ameliorate the infection, and

hopefully. improve osseointegration. This is a difficult problem considering the differences in the composition of cat and human skin and the increased chances for infections in the cat. Although the recent Laminin-5 data (TC-36) seems encouraging, additional animals using this substance will be evaluated to determine whether the application of certain compounds including Laminin-5 or other related growth promoting agents may improve rapid skin attachment to the percutaneous FCS, and possibly improve osseointegration. Although we observed good soft tissue attachment to the BPs in the three cats that received electrostimulation, this procedure did not appear to reduce marsupialization at the level of the FCS. In fact, electrostimulation may have prevented osseointegration in TC-41 by encouraging the ingrowth of fibroblasts under the BP. In future animals, we will pay close attention to the relationship between electrostimulation, skin attachment and osseointegration. Overall, the results of the six animals are encouraging since we have made progress in improving osseointergration and reducing soft tissue infections.

Figure 1. TC-38. An end-portion of the BP(B) is shown attached to the skull surface (arrow). Note the edge of the skull not attached to the BP (arrowheads). A group of connective tissue cells and leukocytes are at the edge of the BP (*). Bar = 1000 µm.

Figure 2. TC-38. Figure 2. Higher magnification of the other edge of the BP (B) shows a mixture of connective tissue cells and leukocytes (*) Bar = 100 µm.

Figure 3. TC-36. This figure shows a cross section of the entire connector including the FCS (F) and the BP (B). Note the epidermis with hair and associated follicles (arrowheads), dermis (D) and subcutaneous tissue (S) all connected to the sides of the FCS and BP. The edge of the skull (arrows) and good osseointegration are shown. Bar = 1000 µm.

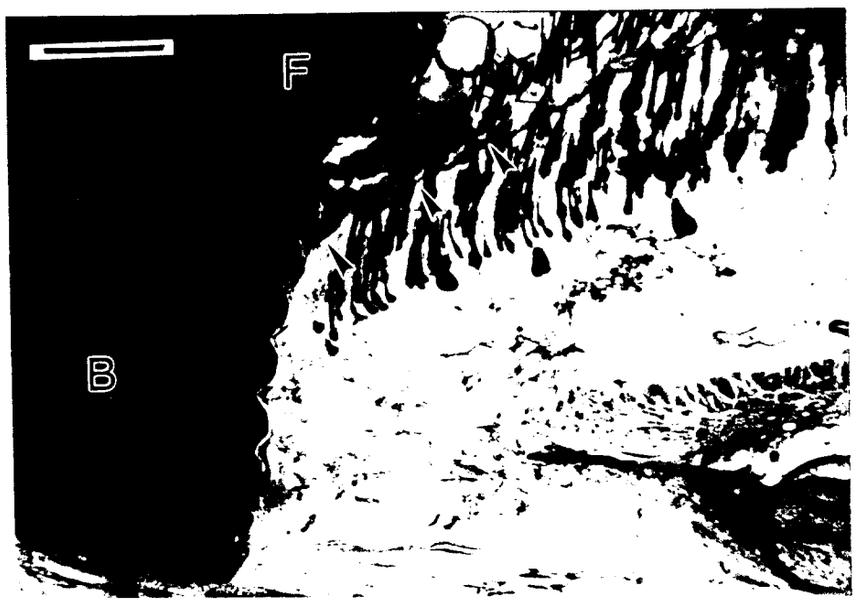


Figure 4. TC-37. The lower edge of the FCS (F) and the side of the BP (B) are shown. Note the marsupialization of the epidermis (arrowheads) curving downward to the side of the BP. Bar = 1000 µm.

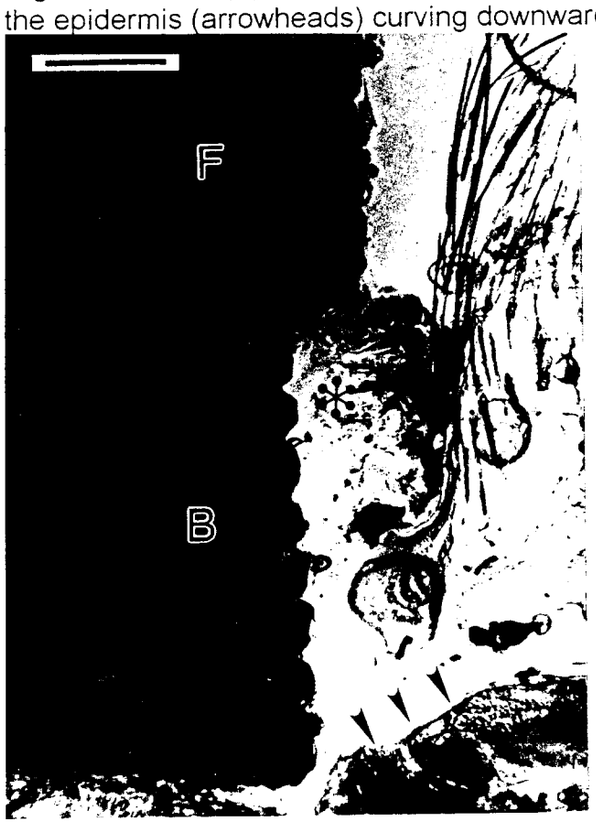


Figure 5. TC-41. This is an example of severe marsupialization in which the entire side of the FCS (F) does not make contact with the skin or subcutaneous tissues. Note the soft tissue (*) extending to the edge of the BP (B). The skull bone is also demarcated (arrowheads). Bar = 1000 µm.

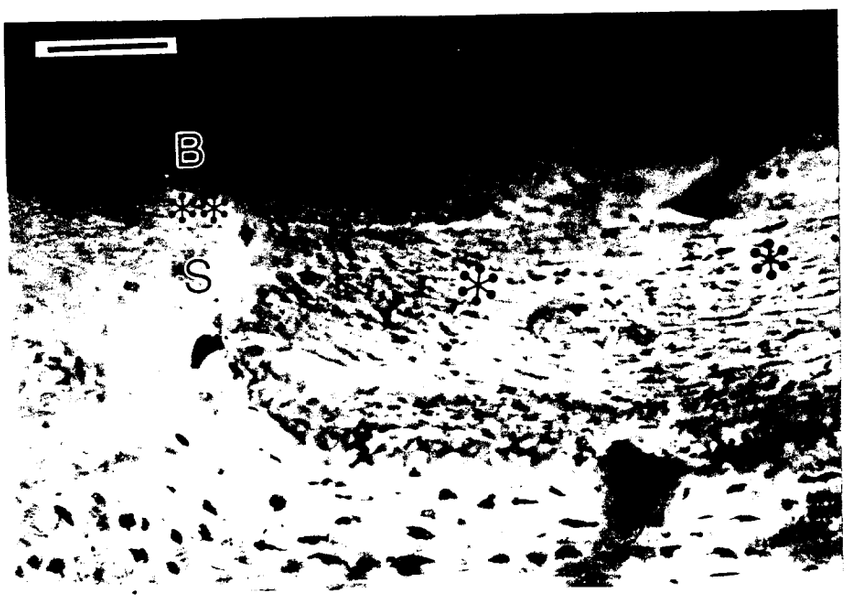
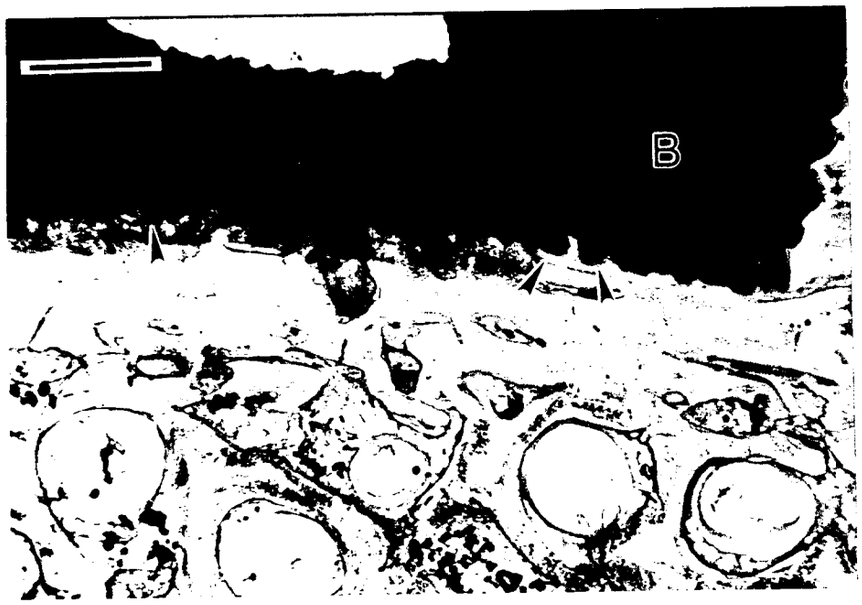


Figure 6. TC-37. The interface between the BP (B) and skull (S) are shown with a large area of connective tissue (*). Osseointegration is good only at the far left portion of the photograph (**). Bar = 100 µm.

Figure 7. TC-40. This figure is similar to Figure 4 and shows a cross section of the entire connector including the beaded-surfaced FCS (F) and BP (B). Note the downward growth of the epidermis (marsupialization - *) and connection of the dermis (D) and subcutaneous tissue (S) only to the side of the BP. Excellent osseointegration of the edge of the BP is shown (**), as is the edge of the skull surface (arrowheads). Bar = 1000 µm.



Figures 8-15 demonstrate sections with good osseointegration. Figure 8. TC-37. Note the excellent bone growth within the titanium beads (arrowheads) on the undersurface of the BP (B). Bar = 1000 µm.

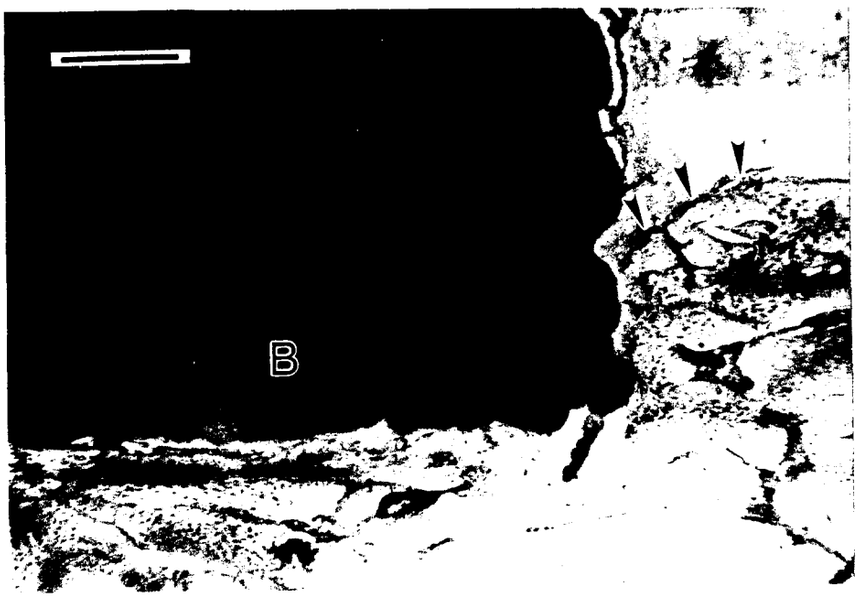


Figure 9. TC-36. Note the excellent osseointegration and the deeply grooved skull surface making for a good seating of the BP (B) onto the skull. Arrowheads delineate the surface of the skull. Bar = 400 µm.

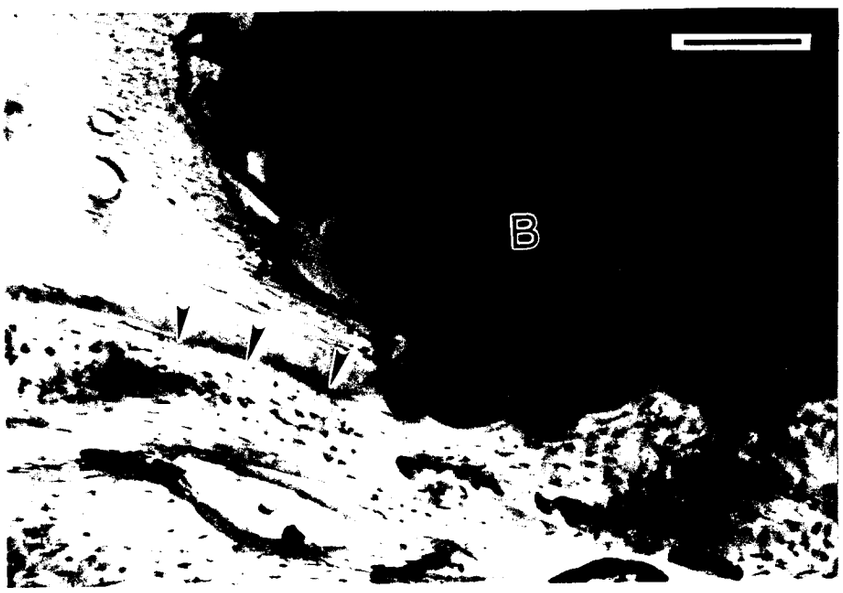


Figure 10. TC-37. The lower edge of the BP (B) is shown with attached connective tissue (*). Although there are gaps between the side of the BP, leukocytes are not present. The skull bone is demarcated by arrowheads. Bar = 200 µm.

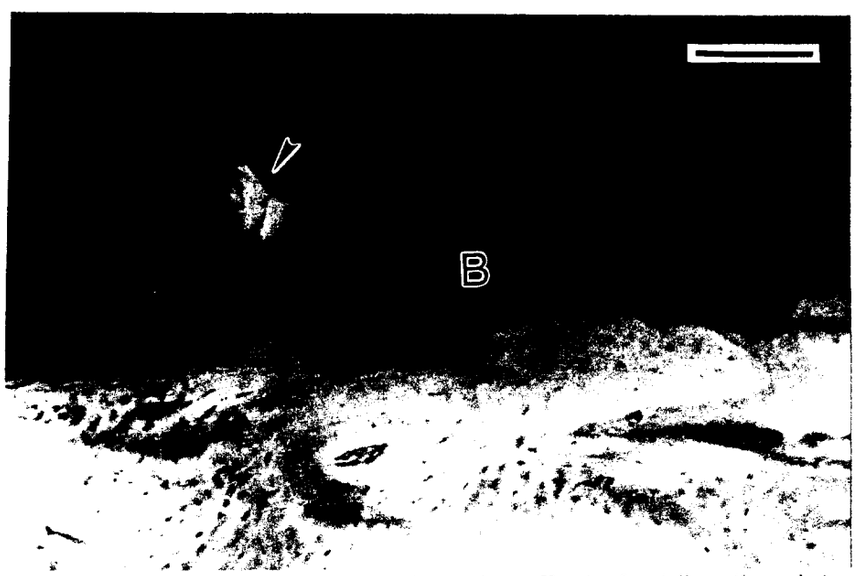


Figure 11. TC-36. This photograph emphasizes the effect of adding the sintered beads to the undersurface of the BP (B). Osteoblasts enter these invaginations (arrowhead), ossify and form strong, dove-tail anchoring points to the metal surface. Bar = 200 µm.

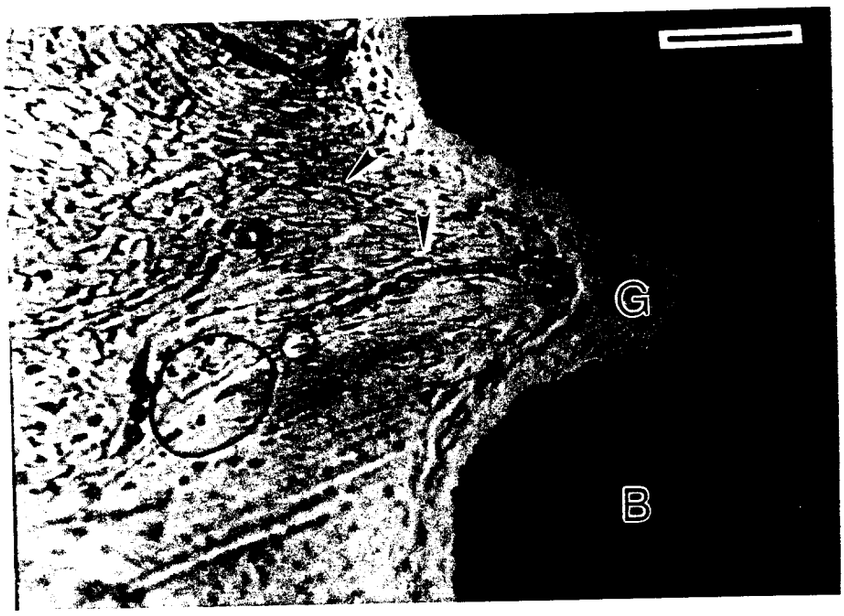


Figure 12. TC-40. This figure is a higher magnification of Figure 7 showing the connective tissue attached to the side of the BP(B). Fibroblasts (arrowheads) appear to be streaming into the machined grooves (G) made in the sides of the BP. Bar = 50 µm.

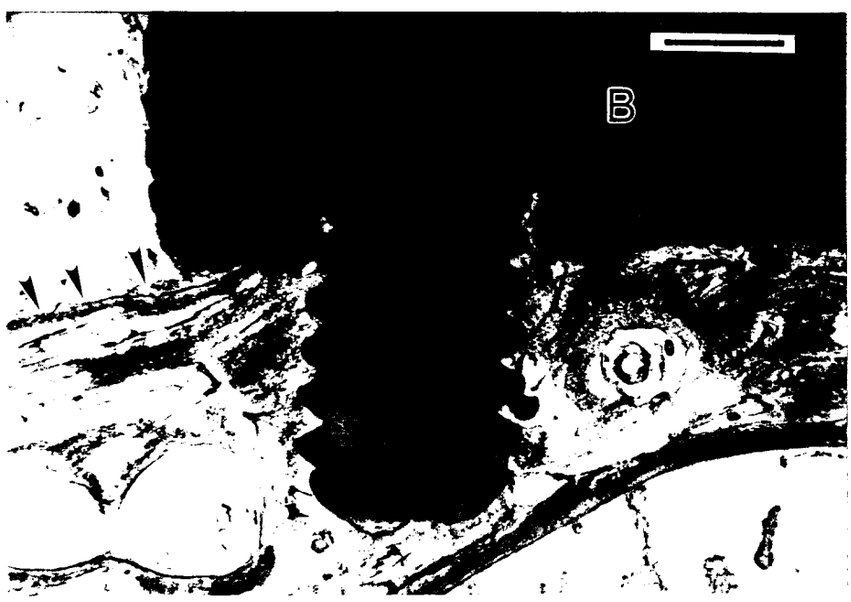


Figure 13. TC-36. A cross section of the edge of the BP (B) and a securing screw are shown. Osseointegration is generally optimum within the deep groves of the screws. The skull bone is delineated with arrowheads. Bar = 1000 µm.

Figure 14. TC-40. The screw in this more frontally positioned connector is shown penetrating into the frontal sinus. A layer of epithelium and osteoblasts have grown over the end of the screw (arrowheads). Excellent osseointegration is also shown at the metal-bone interface. Bar = 1000 µm.

Figure 15. TC-40. The edge of the BP (B) is shown with excellent osseointegration. The edge of the ossified tissue is demarcated by arrowheads. Bar = 400 µm.